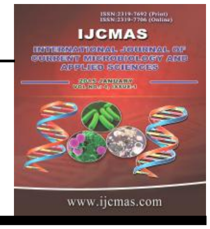
Int.J.Curr.Microbiol.App.Sci (2015) 4(1): 996-1006

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 4 Number 1 (2015) pp. 996-1006
http://www.ijcmas.com



Original Research Article

Topographical distribution of estrogen and progesterone receptors in the endometrium at anterior and posterior uterine walls

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A B S T R A C T

A Cross sectional observational study to examine if there is a statistically significant quantitative difference in the topographical distribution of ER and PR in the endometrium at anterior and posterior uterine walls. Thirty women with male factor of infertility, for whom hysteroscopy was indicated before IVF, were recruited in the study. Hysteroscopic guided punch endometrial biopsies were obtained, during the proliferative phase of the menstrual cycle, from fixed opposing areas at the anterior and posterior endometrial walls near the fundus. Immunohistochemistry (IHC) for estrogen and progesterone receptors was performed using labelled steptavidin–biotin peroxidase-conjugated method. There is no statistically significant difference between the anterior and posterior endometrial walls in staining for ERα or PR as regard glandular cells, stroma cells or total immunopositive cells in functionalis layer of the endometrium. The clinically observed secretory changes at posterior endometrium earlier than anterior might be due to the nongenomic responses to steroids together with variation in the paracrine influence of PR- expressed stromal cells being more at posterior wall that needs to be confirmed by further studies.

Keywords

Estrogen receptors, Progesterone receptors, Endometrium

Introduction

The periodic morphological changes that occur in the endometrium in adult women with regular ovulatory cycles represent one of the most dynamic series of events seen in human tissues (Hayama et al 2002). It is the sex steroid hormones, 17β-estradiol (17β-E2) and progesterone (P), that drive the endometrium through the different phases of the cycle (Talbi et al 2006). These hormones mediate their biological effects on target tissues through gene regulation by nuclear steroid receptors, estrogen receptor (ER) and progesterone receptor (PR), respectively. Also 17β-E2 and P have rapid effects via a variety of signal transduction molecules and pathways that appear to be initiated from the plasma cell membrane(Edwards 2005).

Cellular signaling of estrogens is mediated through two structurally related subtypes of nuclear ER (ERα and ERβ), which are products of different genes located on different chromosomes. Both isotypes bind 17β-E2 with similar high affinity and specificity and both bind the same Estrogen-Responsive Elements (ERE) stimulating transcription. Acting sequentially via their cognate receptors, it is known that 17β-E2 induces and P down-regulates both ERα and PR in human functionalis endometrium. (Pilka et al 2006, Kurita et al 2005)

Thus the expression of endometrial ERα and PR varies temporally across the menstrual cycle.(Critchley et al 2001, Fukunaka et al 2001, Noe et al 1999) Spatially, the topographical distribution and dynamics of ER and PR were studied in radial direction in basalis layer and myometrium (Hayama et al 2002, Fukunaka et al 2001, Noe et al 1999, Al-Hendy et al 2006) as well as along the longitudinal axis of uterus(Al-Hendy et al 2006), in the endocervix(Al-Hendy et al 2006), and in fallopian tubes(Horne et al 2009).

Two clinical observations evoked our interest to study if there is a statistically significant quantitative difference in the topographical distribution of ER and PR between anterior and posterior endometrial walls in normal cycling women. First, the site of implantation and placentation occur in the upper uterine segment (99.5%), more in the posterior surface (2/3) than in the anterior surface (1/3), which accounts for the occipitoanterior being the most common presentation by a fetus that faces the placenta.(Dekel et al 2010, Strowitzki 2006) Second, the observed more obvious secretory changes in the endometrium during hysteroscopy at the posterior wall of the endometrium more evident and precede those in the anterior endometrium during luteal phase in the regularly cyclic women (Inafuku 1992, Sakumoto et al 1992). This study aimed to find if there is a statistically quantitative difference in the topographical distribution of estrogen and progesterone receptors in the human endometrium between the anterior and posterior walls.

Patients and Methods

This pilot prospective analytic study was conducted on thirty consecutive women attending outpatient of Ain Shams University Hospital in the period from February 2013 to 2014. All the studied women were in the reproductive age (23-35years), with regular cycles without gross pelvic pathology and did not receive hormonal treatment in the three months preceding the study. All patients were with male factor of infertility, for which hysteroscopy was indicated as a preliminary step before starting IVF program.

All women were subjected to full clinical evaluation and signed an informed consent for hysteroscopic guided endometrial biopsy. The expression of ER-α and PR in endometrial functional layer were studied during the proliferative phase of the menstrual cycle (from cycle day 6 to 13; mean day 9), when it is known to be at their maximum.(Hayama et al 2002, Pilka et al 2006, Fukunaka et al 2001, Noe 1999, Mertens et al 2001) The study was approved by the The Institutional Review Board of Ain Shams Medical School and was conducted according to the guidelines of the 1975 Declaration of Helsinki on human experimentation.

Endometrial biopsies were obtained by the same investigator, from fixed opposing areas at the anterior and posterior endometrial walls near the fundus using punch biopsy forceps of office hysteroscopy (Karl Storz GmbH & Co. KB). Biopsies were preserved in 10% formalin and labeled Anterior (A) and Posterior (P) for each patient, and sent for histological evaluation and immunostaining. We used monoclonal antibodies raised against the classical ER (ERα) protein. This commercial anti- ERα antibody bound to ERα, but cannot detect ERß.

All endometrial tissue samples were fixed overnight in 10% neutral buffered formalin dehydrated and processed routinely for paraffin embedding. Paraffin blocks were cut into 5 µm thick sections for light microscopical evaluation. One of the serial sections was stained with haematoxylin and eosin for histological evaluation by experienced pathologist to confirm normality and dating of the endometrium (Noyes et al 1950). Abnormal sections (with atypia, in situ neoplasia, endometrial polyps or infection) or those out of the chosen phase of the cycle for sampling were excluded from the study.

Immunostaining

We used monoclonal antibodies raised against the classical ER (ERα) protein. This commercial anti- ERα antibody bound to ERα, but cannot detect ERß. Immunohistochemistry (IHC) for estrogen and progesterone receptors was performed using labelled steptavidin–biotin peroxidase-conjugated method (Vector Laboratories, Inc., Burlingame, CA). For each tissue section, randomly-chosen microscopic fields of cells were viewed under a light microscope at x400 magnifications and photographed using a digital camera to quantify positivity for ER-α and PR respectively using the image analyzer (semiquantitative method) (Figure1). Endometrial glands and stroma were assessed separately excluding surface epithelium and vessel wall if they were encountered in the field. The immunoreactive score (positivity index) for ERα and PR respectively, is expressed as the percentage of immunopositive gland cell nuclei, stroma cell nuclei, and all cell nuclei / field (Figure 2 &3). For each section, more than one field (up to three) were examined by the same observer and the final receptor score was obtained by calculating the arithmetic mean to minimize the bias that might arise from heterogeneity in cell receptor content. In each case, sections from anterior and posterior walls of the normal functionalis proliferative endometrium were compared.

Result and Discussion

In this study, 34 patients were approached of which four cases were excluded (one with fundal Fibroid; one with thick endometrium; one with inadequate endometrial biopsy and one with poor immunstaining). The mean age of the remaining 30 patients was 31.8 years (range: 23-35 years).

Immunohistochemical examination of endometrial biopsies revealed that both ERα and PR were expressed in both the endometrial epithelial and stromal cells and there was no statistical difference in PI for ERα or PR as regard glandular cells, stroma cells or total immunopositive cells in functionalis layer of the endometrium (table I)

Topographical distribution and dynamics of ER and PR are previously studied in radial direction in basalis layer and myometrium(Hayama et al 2002, Critchley et al 2001, Fukunaka et al 2001, Al-Hendy et al 2006) as well as along the longitudinal axis of uterus(Noe et al 1999), in the endocervix(Al-Hendy et al 2006), and in the fallopian tubes(Horne et al 2009) and compared with that observed in functionalis layer of endometrium recovered at similar stages of the menstrual cycle. This pilot study revealed no statistically significant difference between the anterior and posterior endometrial walls in staining for ERα or PR as regard glandular cells, stroma cells or total immunopositive cells in functionalis layer. This lack of spatial or topographic differences of ER and PR is similar to what observed along the longitudinal axis of the uterus and in endocervix.

We used monoclonal antibodies raised against the classical ER (ERα) protein. This commercial anti- ERα antibody bound to ERα, and not ERß. Of note, immunostaining for ERß (wild-type ERβ1) appeared unchanged across the cycle with similar expression in epithelial and stromal cells during both proliferative and secretory phases (Critchley et al 2001).

In this study, IHC reported nuclear localization of ER-α and PR in epithelial and stromal cells with staining for ER-α being more intense than for PR in the proliferative phase and ER-α staining is statistically more intense in the gland than in stroma. Our results are consistent with many reports that studied changes in immunoreactive staining of ER-α and PR in the functional layer across the normal cycle. Both endometrial ERα and PR are up-regulated during the proliferative phase by E2 and subsequently down-regulated in the secretory phase by P.(Edwards 2005, Pilka et al 2006)

The major roles of E2 are for endometrial growth and for priming the endometrium to enable P to act on the tissue. To accomplish the first goal, E2 induces its own ER expression and promotes epithelial and stromal proliferation during the proliferative phase directly through its cognate receptors, and indirectly by induction of growth factors that act as autocrine and/or paracrine modulators. E2 acting via ERα induces proliferation of uterine epithelial cells by the nonclassical tethered pathway independent of binding to classical Estrogen-Responsive Elements (ERE). To accomplish the second goal, E2 induces PR expression thus priming the endometrium for P action. Up-regulation of PR genes by E2 occurs via classical EREs and via tethered pathways and is good evidence of a functional ER-mediated pathway during the proliferative phase of the cycle.(Hayama et al 2002, Critchley et al 2001, O'Brien et al 2006, Petz and Nardulli 2000, Kraus et al 1994, Savouret et al 1994, Savouret et al 1991)

Progesterone is at a maximum concentration in peripheral blood at the mid secretory phase of the cycle when PR in the epithelial cells is waning.(Jabbour et al 2006) In fact, silencing of epithelial PR and ERα coincides with the opening of window of implantation (WOI) and uterine receptivity to implantation (Bazer et al 2010, Macklon et al 2006) and failure of such PR down-regulation is associated with histological delay of the endometrium (a clinically abnormal state).(Lessey et al 1996)

In contrast, stromal cells have high levels of PR in the follicular phase and throughout the luteal phase.(Mylonas et al 2004, Mote et al 2000, Mote et al 1999) PR expression persists in stroma in the upper functional region, being particularly highly expressed in stromal cells in close proximity to uterine vasculature.(Jabbour et al 2006, Bazer et al 2010)

Since Progesterone is the determining hormone of the secretory phase and the stromal cells are the main cell type that retains PR in the secretory phase of the cycle, stromal cells are thought to secrete paracrine signals that convey P actions to the epithelial cells, as well as to endothelial cells and endometrial leukocytes.(Jabbour et al 2006, Mylonas et al 2004, Mote et al 2000, Strowitzki et al 2006, Cunha et al 2004) By late secretory phase, the majority of the glands are negative for PR, thus, the continued P effects on the glands at that time may be mediated by the paracrine influence of PR-expressing stromal cells.(Mylonas et al 2004, Mote et al 2000, Mote et al 1999)

We used a monoclonal antibody that detects a region common for A and B PR isoform. This antibody, thus, identifies total PR but does not specify specific PR isoform. In fact studies reported that PRA and PRB are normally coexpressed with varying ratio in nuclei of PR-positive cells of human endometrium during the menstrual cycle (Mylonas et al 2004, Mote et al 2000, Mote et al 1999).

To standardize endometrial tissue sampling among patients, endometrial biopsies for ER and PR should be performed by the same investigator who take samples from fixed areas of the endometrium during different phases of the cycles which was done in this study (Hayama et al 2002). We studied ERα and PR in women at the childbearing age where the mean age was 31.8 years (range: 23-35 years). It is well known that after menopause, IHC shows maximal constitutive expression for ER and PR in all uterine layers. (Noe et al 1999) These may act as good positive controls for studies of ER and PR in other tissues.

Patients selected for this study had a history of regular menstrual cycles and endometrial tissues were selected in the proliferative phase of the cycle (Noyes et al 1950). Samples that we had used in this study are as normal as we can ascertain.

The presence of uterine fibroids, uterine prolapse or pelvic inflammatory disease may have an impact that confounds normal endometrial receptor expression. In fact, endometrial ER and/or PR expressions are biased in diseases such as endometriosis (Bukulmez et al 2008, Varma et al 2004, Matsuzaki et al 2001, Attia et al 2000) chronic endometritis,(Mishra et al 2008) ectopic pregnancy,(Horne et al 2009) endometrial hyperplasia, (Akesson et al 2010) endometrial polyps,(Gul et al 2010) as well as in malignant human endometrium (Mylonas et al 2004, Balleine et al 2004, Arnett-Mansfield et al 2001, Arnett-Mansfield et al 2004).

The actions of estrogens on the endometrium are mediated by ERα and ERβ; however, the functions of ERß in the uterus are still not fully elucidated. One function of ERß may be to positively or negatively modulate ERα transcriptional activity and would be a key determinant in the differential cellular responses to estrogen and antiestrogens (Jabbour et al 2006, Matthews et al 2006, Matthews and Gustafsson 2003, Matsuda et al 2002, Weatherman and Scanlan 2001, Warnmark et al 2001, Pettersson et al 2000).

Although the immunohistochemical method has been proven sufficient and equivalent to the quantitative assessment by biochemical methods as reported by numerous studies in breast cancer tissue, the concordance between assay results and clinical end points such as disease-free survival, overall survival, and response to endocrine therapies had been extensively discussed. However, it seems that the immunohistochemical steroid receptor analysis is superior to ligand-binding assays as demonstrated for the classic ER(Harvey et al 1999) and PR (Mohsin et 2004) in breast cancer.(Gehrig et al 1999)

Since 1950, the histological point of view has been used for endometrial dating (Noyes et al 1950). Afterwards, the need to understand the genetic mechanism underlying the histological changes emerged. Before the genomic era, researchers were limited to studying "gene by gene" to determine the molecular changes responsible for the alterations observed. However in the "genomic" era, the general trend is a global screening of all the genes transcribed and their interactions. (- Sherwin et al 2006) So in the last decade, the transcriptional mechanisms underlying endometrial biology have been broadly investigated.

However, steroid hormones actions are more complex, they target not only to the nucleus, but also to the cell membrane. By genomic actions, SRs target the nucleus where they act as ligand-dependent TFs, modulating gene expression and protein synthesis with a time lag of hours or even days. However, not all effects of 17β-E2 and P are mediated by direct control of gene expression.

These hormones also exert rapid effects, taking place in seconds or minutes, where they generate a second messenger (cAMP, cGMP) or activate a variety of signal transduction molecules and pathways (kinase activation, and ions flux). In many cases; these effects appear to be initiated from the plasma cell membrane and without the involvement of transcriptional modulation. These rapid responses are referred to as 'non-genomic' or 'extra-nuclear' steroid effects. They are not rare, research has identified many such rapid nongenomic responses to steroids (Edwards 2005, Nourman et al 2004, Losel and Wehling 2003, Flankestein et al 2000)

Table.1 ERα and PR in Anterior versus Posterior endometrium

	Receptors	Anterior	Posterior	P value*
Immuno-positive Gland Cells	**ERα**	92(80.5-98.1)	89.25(80.9-95.5)	0.5025
	PR	63.85(54.375-70.25)	62.35(56.35-68.35)	0.3991
Immuno-positive Stroma Cells	**ERα**	61(58.625-77.9375)	65.25(59.125-80.375)	0.4283
	PR	60.1(52.5-67.4375)	57.5(53.1-62.8125)	0.5170
Total Immuno-positive Cells	**ERα**	76.2375(70.00625-84.575)	79.125(72.25-83.76875)	0.7734
	PR	61.975(59.0625-68.9625)	61.525(57.2875-66.925)	0.4405

Values are given as median & interquarntile range
* Wilcoxon signed rank test

Figure.1 The screen of the image analyzer during counting the percentage of positive glandular cell nuclei

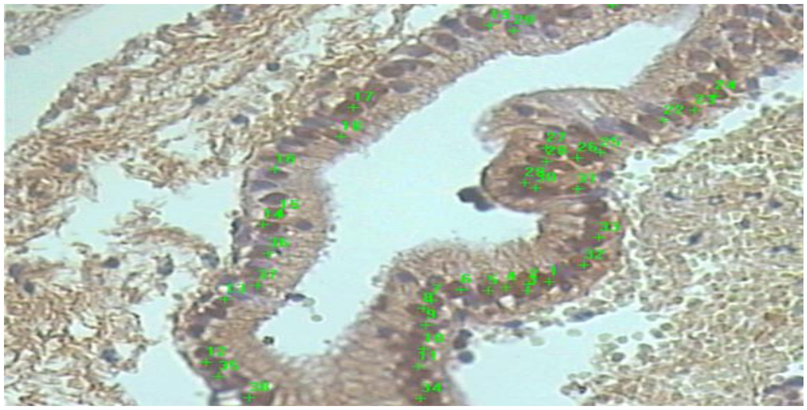


Figure.2 Immunostaining for estrogen receptor in endometrium of anterior uterine wall showing positive nuclear staining of both glands (black arrow) and stroma (white arrow) (X400)

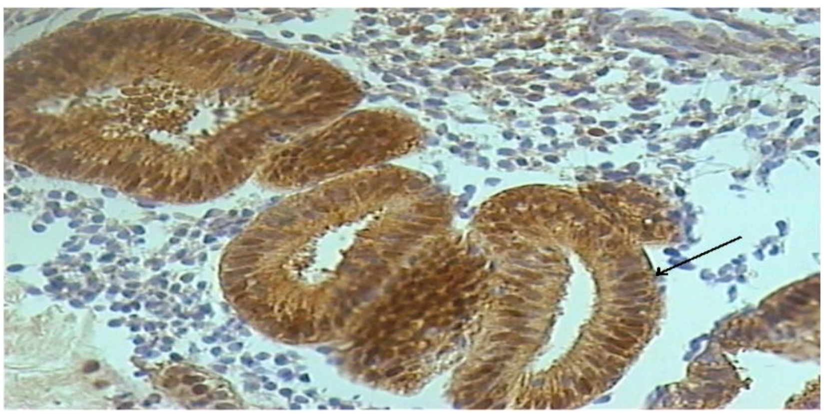
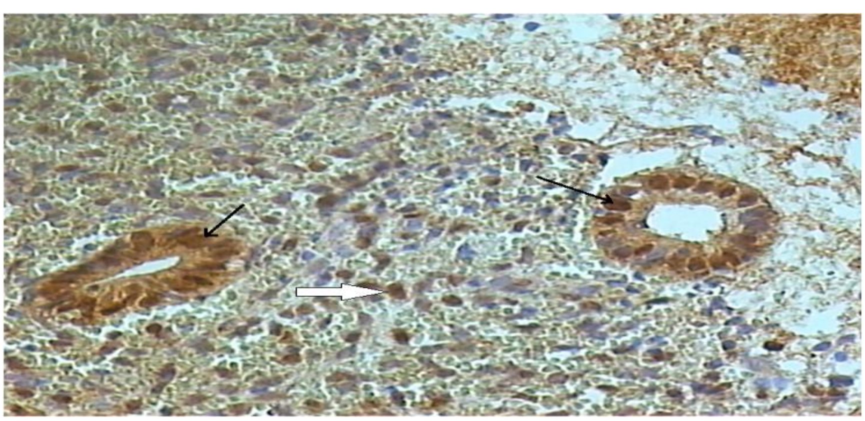


Figure.3 Immunostaining for progesterone receptor in endometrium of posterior uterine wall (X400)



Acknowledgement

The Authors would like to thank Dr Sahar Samy Ezz-Elarab *(M.B,B.Ch, M.Sc, MD. Public Health, Early Cancer Detection Unit-Obst & Gyn Hospital, Ain Shams University Hospitals, Mobile 0101438659)*, for her statistical assistance.

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